題目　肝機能障害の評価法とその障害機序に関する研究；
ヒト肝ミトコンドリアの日内代謝変動に基づく肝機能評価
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# Diurnal Fluctuations of Arterial Ketone Body Ratio in Normal Subjects and Patients With Liver Dysfunction

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**To explore the metabolic aspects of chronic liver disease, diurnal changes of arterial ketone body ratio (acetoacetate/3-hydroxybutyrate), reflecting hepatic mitochondrial redox potential, were investigated in normal subjects, patients with chronic liver disease (Child's class A or B), and patients with hepatic failure (Child's class C). Ketone body ratio in normal subjects increased after breakfast from 0.96 to 2.00, after lunch from 2.17 to 2.38, and after dinner from 1.23 to 2.55 with blood glucose level ranging from 103 to 141 mg/dL (5.7 to 7.8 mmol/L). By contrast, the ketone body ratio in the Child A or B group changed little and remained within a range of 0.70–1.35 despite a large change in blood glucose level from 102 to 176 mg/dL (5.7 to 9.8 mmol/L). Ketone body ratio in Child's class C remained near or below 0.4 with no response to glucose administration, despite a marked elevation in blood glucose level. These results indicate that hepatic mitochondrial redox potential undergoes diurnal changes in sharp response to meals in normal liver function but that these fluctuations are absent in patients with liver damage (Child's class A, B, and C). Furthermore, it remains at low levels in severe liver failure (Child's class C). It is also suggested that hepatic mitochondrial redox potential plays an important role in the regulation of blood glucose levels.**

Since we proposed the redox theory in the early 1980s (1,2), our research has been focused on the essential role of arterial ketone body ratio (AKBR; acetoacetate/3-hydroxybutyrate) in energy metabolism as the metabolic basis of multiple systemic organ failure (3,4). Experimental findings indicate that AKBR is an accurate parameter to estimate the hepatic function in primary liver disease as well as in hepatectomy, hypoxia, hemodilution, and hemorrhagic shock (5–9). The redox theory is now receiving wider acceptance, and AKBR has begun to be applied clinically to evaluate the changes in hepatic function (10–13). More recently, AKBR has been reported to have potential use as an early indicator of graft failure after human liver transplantation (14,15).

The AKBR decreases in patients with liver disease, and it remains below 0.4 in patients with severe hepatic failure, at which level it hardly responds to any form of intensive treatment. Eighty-five percent of the patients with AKBR below 0.4 die of liver failure complicated by multiple organ insufficiency (1,2,11). On the other hand, the AKBR in the normal liver rapidly responds to factors such as hepatic blood flow, glucose load, and insulin and attains high levels over 0.7 (16,17).

This study aims to clarify the extent of fluctuation in AKBR in the cycle of daily life. The diurnal changes in AKBR were measured in normal subjects, patients with chronic liver disease, and patients with severe hepatic failure. Our findings show that a different pattern in diurnal changes of AKBR exists between normal and impaired livers and that diurnal changes in AKBR have a tendency to flatten and remain at low levels in accordance with the severity of liver dysfunction.

## Materials and Methods

This study was performed in six young male volunteers as a control group (group 1), seven patients with chronic liver disease (group 2), and three patients with severe hepatic failure (group 3) under informed consent

*Abbreviations used in this paper:* AKBR, arterial ketone body ratio; TCA, tricarboxylic acid.



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主論文2

# Alterations in the proton ATPase activity of rat-liver mitochondria after hemorrhagic shock

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## ABSTRACT

To clarify the damage site of complicated oxidative phosphorylation function after hemorrhagic shock in jaundiced liver mitochondria, the H+-ATPase activity of inside-out submitochondrial particles, mitochondrial membrane potential, and oxygen consumption in the presence of uncoupler were studied as indices of phosphorylation, membrane intactness, and oxidation, respectively. Hemorrhagic shock was induced according to the Wiggers' model (mean arterial blood pressure=40 mmHg) in rats made jaundiced by common bile duct ligation; sham-operated rats served as controls. After reinfusion of the shed blood, all of the control rats survived but all of the jaundiced rats died. Liver mitochondria from jaundiced rats after 1 hr hypotension demonstrated a 48% decrease in mitochondrial ATPase activity without remarkable changes in either oxidative activity or membrane potential of liver mitochondria. The reduction of ATPase activity appeared to be due to its release in the supernatants obtained from submitochondrial particles since the ATP activity of supernatants in jaundiced rats was significantly ($p<0.001$) higher than that of the controls. It is suggested that this enzyme plays a key role in energy restoration in recovery from shock.

**Running head**: Proton ATPase in shock

**Abbreviations**: ADP = adenosine diphosphate; ATP = adenosine triphosphate; ESMP = submitochondrial particles prepared in the presence of ethylenediaminetetraacetic acid; $F_o$ = membrane integral sector of mitochondrial proton adenosinetriphosphatase complex; $F_1$ = catalytic part of mitochondrial proton adenosinetriphosphatase complex; FCCP = carbonyl cyanide p-trifluoromethoxyphenylhydrazone; H+-ATPase = proton adenosinetriphosphatase complex. SDS = sodium dodecyl sulfate; PAGE = polyacrylamide gel electrophoresis

## INTRODUCTION

Many clinical studies have demonstrated that operation on severe obstructive jaundice is associated with a postoperative morbidity of 40-60 % and a mortality of 15-25 %.[1-6] Postoperative complications and causes of death include renal and hepatic failure, infection and hemorrhage. Bile duct ligation induces many biological alterations including hemodynamic and metabolic abnormalities.[7-10] Once metabolic functions of the liver are impaired with biliary obstruction, the impairment takes six weeks or more to improve after release of obstruction.[10] Experimental animals with obstructive jaundice have an increased susceptibility to hemorrhagic hypotension[7,8] and have an increased tendency to develop shock following hemorrhage.[11] Although some studies deal with the hemodynamic abnormalities in these animals, the metabolic aspects of obstructive jaundiced liver during hemorrhagic shock have not been well documented. Previous reports[12,13] from our laboratory showed that hemorrhagic shock for two hours in obstructive jaundiced animals proved lethal and that severe impairment of mitochondrial oxidative phosphorylation was observed. The authors thought that poor recovery of energy state

after reinfusion of the reserved blood due to the impairment of oxidative phosphorylation may be related to so-called irreversible shock. It has been reported that the decline in ATP levels or energy state is a critical event in the development of cell damage.[14,15] Although it may be controversial whether abnormalities in ATP levels and ATP production have relevance for the high mortality rate, low ATP levels at least lead to inactivation of energy-requiring systems. Maintenance of ATP levels or ATP production systems is thought be important for critical cellular functions.[16,17]

Oxidative phosphorylation should be functionally and structurally separable into oxidative proton translocating and phosphorylating proton translocating complexes, namely electron transport proteins and H+-ATPase, respectively.[18] These two systems are coupled to produce ATP by means of an electrochemical proton gradient.[19] In this study, in order to clarify the damage site of complicated oxidative phosphorylation system using obstructive jaundiced rats subjected to hemorrhagic shock, the H+-ATPase activity of inside-out submitochondrial particles and oxygen consumption of mitochondria in the presence of an uncoupler were

studied as indices of phosphorylation and oxidation, respectively. It is also important to determine whether the mitochondrial membrane is intact since membrane permeability greatly affects oxidative phosphorylation. Therefore the mitochondrial membrane potential was measured as an indicator of membrane intactness.

Evidence will be presented indicating that the disability of phosphorylation occurred in jaundiced liver mitochondria at 1 hr after hemorrhagic shock. H+-ATPase is comprised of the membrane sector ($F_o$) and the catalytic part ($F_1$). The $F_1$ consists of the five subunits ($\alpha$, $\beta$, $\gamma$, $\delta$, and $\varepsilon$ in order of decreasing molecular weight). It is known that only the $\alpha$ and $\beta$ subunits bind ATP and that $\beta$ subunit is a catalytic center of ATPase. Molecular changes in H+-ATPase were also studied by oligomycin sensitivity of ATPase and SDS-PAGE analysis of submitochondrial particles.

## METHODS

Male albino rats of Wistar strain were maintained on Clea 2 (Nippon Haigoshiryo Co Ltd., Osaka, Japan) and water *ad libitum* for two weeks before the operation. Those weighing about 150 grams

were divided into two groups, jaundiced rats and sham operated rats. Jaundice was produced by common bile duct ligation under ether anesthesia. Food was withheld for 15 hours pre- and postoperatively. The bile duct was doubly ligated just distal to the junction of the hepatic ducts with 5-0 nylon suture without damage to the pancreatic duct. Sham-operated rats were subjected to the same treatment without common bile duct ligation.

Ten days later both jaundiced and control rats increased body weight to about 200 grams. After being fasted for 15 hours, they were anesthetized with an intraperitoneal injection of 25 mg of pentobarbital sodium per kilogram of body weight and fixed on a surgical board in the supine position. A 26 gauge plastic cannula inserted into the femoral artery was connected to a transducer and to a plastic syringe and blood reservoir. Three hundred units of heparin sodium were administered immediately after cannulation. After respiration and blood pressure were confirmed to be stable, hemorrhagic shock was induced by withdrawing blood via the heparinized syringe until the mean arterial blood pressure fell to 40 mmHg. For the following 1-2 hr, it was necessary to continue extracting or infusing small amounts of blood to maintain a

constant mean arterial blood pressure.[20] After 1 or 2 hr hypotension, all reserved blood was returned to the rats. At 24 hr after the induction of shock, the survival rate was 0% (0/10) in jaundiced rats, but 100% (10/10) in control rats. All procedures were performed under conditions as sterile as possible. All experiments involving animals were carried out in accordance with the institution's guidelines for the care and use of laboratory animals.

Liver mitochondria were prepared after 1 or 2 hr hypotension by the method described previously.[21]

Oxidative phosphorylation function was monitored polarographically by the conventional method previously reported.[22]

Oxidative activity was evaluated polarographically by measuring oxygen consumption rate in the presence of FCCP as an uncoupler. Assay for oxidative activity was done at 22°C (pH 7.4) in a medium containing 0.3 M mannitol, 10 mM potassium chloride, 2 mM magnesium chloride, 10 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer, 3 mM potassium phosphate buffer, and 0.2 mM ethylenediaminetetraacetic acid. Glutamate as a substrate was added at a concentration of 3 mM and FCCP was added at concentration of 1

μM. The oxygen consumption rate in the presence of FCCP was calculated from the polarographic tracings.

Mitochondrial membrane potential was measured by using FCCP according to the method of Akerman[23] which was modified as follows. The reaction medium contained 0.2 M mannitol 0.38 mM ethylenediaminetetraacetic acid, 20 mM N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid (pH 7.4 with a tris(hydroxymethyl)aminomethane buffer) and 20 mM potassium chloride in 3 ml final volume. Safranine O was used in a final concentration of 9.6 μM. The reactions were started by addition of freshly isolated mitochondria to reaction medium containing safranine O, with (state 4) or without (state 1) addition of glutamate as substrate. When absorbance change reached equilibrium, 0.48 μM FCCP was added, which resulted in a rapid absorbance change. Reactions were performed in cuvettes of 1cm light path at 22°C. Changes in absorption spectra were recorded with a two-wave length double beam spectrophotometer (UV 3000, Shimadzu Co Ltd., Kyoto, Japan) set at 511 to 533 nm. All measurements were done in triplicate. The membrane potential was calibrated by the potassium diffusion potential. Valinomycin induced potassium diffusion potential was

calculated from the Nernst equation using values of $[K^+]_i$ and $[K^+]_o$ in the presence of valinomycin: $dE=60 \times \log[K^+]_i/[K^+]_o$ where dE is the membrane potential in millivolts, and $[K^+]_i$ and $[K^+]_o$ represent the internal (matrix) and external (medium) concentrations of potassium ion, respectively, with $[K^+]_i$ assumed to be 120 mM.[23]

Preparation of ESMP and determination of ATPase activity were performed as followed. Purified submitochondrial particles were prepared by a procedure modified from that of Lee et al..[24] Freshly isolated mitochondria were stored for 7 days at -30°C and thawed immediately before use. They were suspended at a concentration of 20 mg protein/ml in 0.3 M mannitol, containing 2 mM ethylenediamine-tetraacetic acid (final pH 8.5), and were then exposed to ultrasonic energy for 60 s at 4°C (Heat Systems Ultrasonics, Model W-225, output 70 W). Large mitochondrial fragments were removed by centrifugation at 10000g for 10 min. Submitochondrial particles were sedimented from the resulting supernatant at 100000 g for 40 min at 4°C. The sediment was resuspended to a final concentration of 30 mg/ml with 0.3 M mannitol. ATPase activity was determined spectrophotometrically at 340 nm in a thermostatically controlled reaction cuvette at 30°C by

coupling the production of ADP to the oxidation of reduced nicotinamide adenine dinucleotide via the pyruvate kinase and lactate dehydrogenase reactions by a modified method of Buckle et al..[25] The reaction mixture contained 0.3 M mannitol, 50 mM potassium chloride, 5 mM magnesium chloride, 20 mM tris(hydroxymethyl)-aminomethane hydrochloride pH 7.4, 0.5 μg/ml rotenone, 0.1 mM reduced form of nicotinamide adenine dinucleotide, 1 mM phosphoenolpyruvate, 5 units/ml lactate dehydrogenase, 2 units/ml pyruvate kinase and 20-30 μg/ml submitochondrial particles in a final volume of 3 ml. The reaction was started by the addition of ATP at the concentrations of 0.025 to 1.6 mM. The data were analyzed based on the Michaelis-Menten equation to give Vmax and Km. The oligomycin sensitivity of the ATPase activity was measured over the range from 1.0 to 20 nmol of oligomycin.

SDS-PAGE was carried out on 12.5% polyacrylamide gels in the SDS and 3.2 mM urea.[26] Western blotting and subsequent immunodecoration were performed as described previously utilizing an anti-bovine heart mitochondrial $F_1$ antibody.[27] Quantitative measurements of $F_1$ subunits from Western blots were performed using Soft Laser Scanning Densitometer (Model SLR 20/10 Biomed Instrument, Inc., Fullerton,

CA, U.S.A.). Protein concentrations were measured by the method of Lowry et al. using bovine serum albumin as standard.[28]

Safranine O was obtained from Chroma Gesellschaft Schmid & Co., FCCP from Fluka A.G., phosphoenolpyruvate, nicotinamide adenine dinucleotide reduced form, valinomycin and oligomycin from Sigma Chemical Co., lactate dehydrogenase and pyruvate kinase from Boehringer (Mannheim). Anti-bovine heart mitochondrial $F_1$ antibody was generous gift from Dr. Yasuo Kagawa of Jichi Medical School, Tochigi, Japan. Other reagents were of the highest grade commercially available.

All results are expressed as mean $\pm$ SEM. Statistical analysis was made with analysis of variance and Student's t-test. Statistical significance was obtained when the P value was less than 0.05.

## RESULTS

Table 1 shows the respiratory control ratio, ADP/O ratio, state 3 respiratory rate, and phosphorylation rate after induction of hemorrhagic shock in control and jaundiced rats. The respiratory control ratio with glutamate or succinate as substrate significantly decreased after 1 and 2 hr hypotension in jaundiced

rats. Coupling of oxidation and phosphorylation is judged by ADP/O ratio and ADP/O ratio did not decrease after 1 or 2 hr hypotension in controls. By contrast, in jaundiced rats, it did not decrease after 1 hr hypotension but decreased significantly after 2 hr hypotension. The state 3 respiratory rate (n mole of atom oxygen per min per mg of mitochondrial protein) and the phosphorylation rate (n mole of ATP synthesized per min per mg of mitochondrial protein) with glutamate or succinate as substrate decreased significantly after 1 and 2 hr hypotension in jaundiced liver mitochondria. From the above results, the oxidative phosphorylation activity decreased significantly after hemorrhagic shock in jaundiced rats.

Table 2 shows the changes in respiration rate of liver mitochondria in the presence of FCCP after induction of hemorrhagic shock in control and jaundiced rats. The FCCP-stimulated respiration rate with glutamate as substrate tends to decrease at 2 hr after induction of shock in jaundiced rats but has no significant decrease. The uncoupler stimulated respiration rate with succinate has no variations even after induction of shock in control and jaundiced animals. These observations imply

that no remarkable damage occurs to the electron transport system.

Table 3 shows the mitochondrial membrane potential after induction of hemorrhagic shock in control and jaundiced rats. Membrane potential in state 1 and state 4 had no decrease during hemorrhagic shock in jaundiced and control rats. This result indicates that the mitochondrial membrane was intact during the observation period.

Table 4 shows H+-ATPase activity after induction of hemorrhagic shock in control and jaundiced rats. There was no significant difference in the mean values of Vmax and Km from ESMP between control and jaundiced rats before shock and their values were consistent with those of a previous report.[25] By contrast, H+-ATPase activity was drastically lowered by 48% in ESMP from jaundiced rats after 1 hr hypotension, and by 72% after 2 hr hypotension. These results indicate that phosphorylative activity decreases after shock in jaundiced animals. Km increased in jaundiced rats after shock.

SDS-PAGE was performed to examine the molecular changes in five subunits of $F_1$. As shown in Figure 1, SDS-PAGE followed by Western blotting with anti-bovine heart mitochondrial $F_1$ antibody indicated

that there was no change in the $\alpha/\beta$ ratio in pure $F_1$, control and jaundiced ESMP after 1 hr hypotension. Densitometrical data of $\alpha/\beta$ ratio were 1.01, 0.98, and 1.00, in purified $F_1$, control, and jaundiced ESMP, respectively. This indicates that we can not account for the reduction of ATPase activity observed in jaundiced mitochondria after shock by dissociation of $\alpha$ or $\beta$ subunit from $F_1$. Because $\gamma$, $\delta$ and $\varepsilon$ subunits are small proteins which are not as easily detected as $\alpha$ and $\beta$ subunits, we have no information concerning changes of these subunits.

We examined oligomycin sensitivity and H+-ATPase activity of supernatants obtained from ESMP. $F_o$ of H+-ATPase is a water-insoluble component that spans the energy transducing membrane and directs protons toward or from $F_1$, a water-soluble catalytic component. It is known that H+-ATPase activity is inhibited by oligomycin and also that oligomycin sensitivity is diagnostic of a correct assembly of $F_1$ to $F_o$.[18] As shown in Table 5, oligomycin sensitivity was significantly lowered in ESMP from jaundiced rats after shock. ATPase activity of supernatants in jaundiced rats after shock was significantly higher than that of

the controls. This suggests that a significant portion of the $F_1$ was solubilized from jaundiced mitochondria after shock. On the other hand, only a small portion of $F_1$ was released from control mitochondria.

## DISCUSSION

The present study demonstrates that oxidative phosphorylation is more significantly impaired in jaundiced rats subjected to hemorrhagic shock than in controls and that this is to be due to a defect in the terminal phosphorylation phase of oxidative phosphorylation rather than a defect in the electron transport chain. As an important control, we show no change in liver mitochondrial membrane potential suggesting that the mitochondrial membrane remained intact during the period of hemorrhagic shock. H+-ATPase is more vulnerable to the combined stresses of obstructive jaundice and hemorrhagic shock, than respiratory chain and mitochondrial membrane. A recent paper has indicated that the membrane potential is maintained for a comparative long period during mitochondrial injury and breaks down rather promptly beyond

a critical point.[29] An other paper reported that ATPase activity decreased most rapidly compared with the activities of mitochondrial inner membrane enzyme complexes of electron transport in rat ischemic heart.[30] Experimental results obtained from our model are compatible with these papers.

The reduction of ATPase activity appears to be due to considerable release of $F_1$ into the supernatant. The difference in oligomycin sensitivity between control and jaundiced mitochondria after shock indicates that there may be altered assembly of $F_1$ to $F_o$ which interacts with oligomycin.[18] Our observation demonstrates that the water-soluble catalytic subunits, $F_1$, are not as tightly bound to the inner membrane as in control mitochondria. This loss in affinity suggests the possibility that the function of proteins thought to make structural links between $F_1$ and $F_o$ has been compromised in jaundiced mitochondria after hemorrhagic shock. The properties of the $F_1$ portion may be changed by perturbation of the membrane sector subunits, causing the impairment of phosphorylation, as supported by other investigators.[31,32]

It has been reported that an intrinsic inhibitor protein which binds to the H+-ATPase exists in mitochondria.[33,34] If the

inhibitor protein increases after hemorrhagic shock, especially in jaundiced liver, H+-ATPase activity may decrease. However, it is unlikely that the inhibitor protein can increase sufficiently to cause an effect after 1 or 2 hr of shock, especially only in jaundiced liver. Furthermore, if the inhibitor protein dissociates from ATPase, ATPase activity increases. But this study indicates a decrease of ATPase activity in jaundiced rats after shock. Therefore, there is little possibility in this case that the inhibitor protein is important in H+-ATPase activity.

Common bile duct ligation induces alterations in many biological phenomena, especially the elevation of bilirubin and bile acid levels which have cytotoxic effects. Since it is known that bile acids have an especially high hepatotoxic potential as detergents,[35,36] these bile acids may be effective in dissociation $F_1$ from $F_o$. When ATP production decreases due to disability of phosphorylation, intracellular calcium increases because the Ca pump is energy-dependent. Calcium enhances the detergent activity of bile salts[35] and the decrease of ATPase activity may be accelerated more.

Bilirubin has been shown to have an inhibitory effect on

membrane-requiring mitochondrial reactions.[37] More recent study suggests that the binding site of an organic anion such as bilirubin is present on β subunit of $F_1$.[38] Bilirubin is known to uncouple oxidative phosphorylation in mitochondria.[39] This study shows no changes before shock and after 1 hr hypotension in ADP/O ratio which is an index in judging coupling of oxidation and phosphorylation. Therefore, there may be little effect of bilirubin as a uncoupler by the period of at least first 1 hr hypotension. Tissue and albumin compete for binding the body's bilirubin pool. It has been reported that the binding of bilirubin to mitochondria is not determined by the free bilirubin level, but by the concentration of the pH dependent subfraction, the free bilirubin acid salt.[40] Since the uptake of bilirubin by mitochondria increases at lower pH and since acidosis is profound in the cell and the extracellular fluid in hemorrhagic shock, the cytotoxicity of bilirubin will increase during hemorrhagic shock. In conclusion, the activity of H+-ATPase decreases significantly by hemorrhagic shock in jaundiced rats without remarkable changes in either the oxidative activity or the membrane potential of liver mitochondria. This decrease of activity may be explained by

the possibility that the binding $F_1$ to $F_o$ is compromised. It is suggested that the $F_oF_1$-ATPase complex plays key role in energy restoration in recovery from shock.

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## Table I.

Changes in respiratory control ratio, ADP/O ratio, state 3 respiration, and phosphorylation rate after induction of hemorrhagic shock in control and jaundiced rats.

Glutamate as substrate

| | RC | State 3 (nO/min per mg) | ADP/O | PR (nATP/min per mg) |
|---|---|---|---|---|
| Control (Sham) | (6) 5.72±0.07 | 44.2±1.8 | 2.77±0.04 | 122.1±3.7 |
| After induction of hemorrhagic shock | | | | |
| 1hr | (8) 5.97±0.33 | 44.8±1.2 | 2.72±0.03 | 121.6±2.5 |
| 2hr | (6) 5.76±0.22 | 44.1±1.6 | 2.76±0.03 | 121.5±4.0 |
| Jaundiced (CBL) | (6) 5.86±0.26 | 44.5±2.6 | 2.87±0.04 | 127.3±2.3 |
| After induction of hemorrhagic shock | | | | |
| 1hr | (8) 4.85±0.10[a] | 37.6±1.2[b] | 2.77±0.04 | 104.1±2.6[c] |
| 2hr | (6) 2.98±0.15[c] | 26.5±1.5[b] | 2.29±0.06[a] | 60.4±3.1[c] |

(continued)

Succinate as substrate

| | | RC | State 3 (nO/min per mg) | ADP/O | PR (nATP/min per mg) |
|---|---|---|---|---|---|
| Control (Sham) | (6) | 5.16±0.22 | 95.0±2.8 | 1.84±0.07 | 173.9±5.4 |
| After induction of hemorrhagic shock | | | | | |
| 1hr | (8) | 5.05±0.12 | 98.4±2.4 | 1.83±0.04 | 179.9±5.9 |
| 2hr | (6) | 4.81±0.12 | 96.6±3.9 | 1.74±0.03 | 167.6±6.9 |
| Jaundiced (CBL) | (6) | 4.80±0.08 | 96.7±3.3 | 1.79±0.04 | 172.8±12.7 |
| After induction of hemorrhagic shock | | | | | |
| 1hr | (8) | 3.73±0.18[c] | 82.8±3.7[b] | 1.74±0.06 | 142.7±4.3[c] |
| 2hr | (6) | 2.36±0.10[c] | 67.1±5.6[b] | 1.46±0.13[a] | 95.9±5.8[c] |

Values are means ± standard error of mean; number of rats is shown in parentheses.

The abbreviations used are: RC = respiratory control ratio; State 3 = state 3 respiration (n mole of atom oxygen per min per mg of mitochondrial protein); ADP/O = mole of ATP synthesized per mole of atom oxygen consumed; PR = phosphorylation rate (n mole of ATP synthesized per min per mg of mitochondrial protein)

Sham = sham operation, CBL = common bile duct ligation.

[a] $P<0.05$ compared with control groups

[b] $P<0.01$ compared with control groups

[c] $P<0.001$ compared with control groups

## Table II.

Changes in respiration rate of liver mitochondria in the presence of FCCP after induction of hemorrhagic shock in control and jaundiced rats. ---- Oxidative activity of liver mitochondria

| Substrate | Glutamate (nO/min per mg) | Succinate (nO/min per mg) |
|---|---|---|
| Control (6) (Sham) | 53.4±1.7 | 144.7±5.9 |
| After induction of hemorrhagic shock | | |
| 1hr (8) | 55.2±2.6 | 144.7±5.0 |
| 2hr (6) | 52.4±3.7 | 138.9±8.2 |
| Jaundiced (6) (CBL) | 54.6±6.6 | 138.4±1.4 |
| After induction of hemorrhagic shock | | |
| 1hr (8) | 52.2±2.6 | 136.7±3.7 |
| 2hr (6) | 45.4±2.3 | 139.6±5.8 |

Values are means ± standard error of mean; number of rats is shown in parentheses. The abbreviations used are: Sham = sham operation, CBL = common bile duct ligation.

## Table III.

Changes in membrane potential of liver mitochondria after induction of hemorrhagic shock in control and jaundiced rats.

| | | state 1 (mV) | state 4 (mV) |
|---|---|---|---|
| Control (Sham) | (4) | 124±5 | 152±8 |
| After induction of hemorrhagic shock | | | |
| 1 hr | (8) | 126±5 | 152±5 |
| 2 hr | (4) | 117±6 | 145±6 |
| Jaundiced (CBL) | (4) | 128±7 | 155±5 |
| After induction of hemorrhagic shock | | | |
| 1 hr | (6) | 127±5 | 153±6 |
| 2 hr | (4) | 128±5 | 165±5 |

Values are means ± standard error of mean; number of rats is shown in parentheses.

The abbreviations used are: Sham = sham operation, CBL = common bile duct ligation.

## Table IV.

Changes in ATPase activity after induction of hemorrhagic shock in control and jaundiced rats

| | | Vmax (μmol/min per mg) | Km (mM ATP) |
|---|---|---|---|
| Control (Sham) | (6) | 1.16±0.02 | 0.20±0.05 |
| After induction of hemorrhagic shock | | | |
| 1hr | (8) | 1.18±0.06 | 0.21±0.02 |
| 2hr | (6) | 1.16±0.07 | 0.20±0.05 |
| Jaundiced (CBL) | (6) | 1.14±0.09 | 0.19±0.03 |
| After induction of hemorrhagic shock | | | |
| 1hr | (8) | 0.61±0.02[a] | 0.26±0.02[b] |
| 2hr | (6) | 0.32±0.05[a] | 0.27±0.04[b] |

Values are means ± standard error of mean; number of rats is shown in parentheses.

The abbreviations used are: Sham = sham operation, CBL = common bile duct ligation

[a] $P<0.001$ compared with control groups.

[b] $P<0.02$ compared with control groups.

## Table V.

H+-ATPase activity of liver mitochondria at 1 hr after induction of hemorrhagic shock in control and jaundiced rats. (Oligomycin sensitivity and affinity of $F_1$ for the inner membrane)

| Fraction[a] | ATPase activity | |
|---|---|---|
| | total units[b] | oligomycin sensitivity |
| ESMP | | |
| Control | 100±6 | 88±1 |
| Jaundiced | 52±4[c] | 67±1[c] |
| Supernatant from ESMP preparation | | |
| Control | 4±1 | 78±3 |
| Jaundiced | 34±2[c] | 16±1[c] |

Values are means ± standard error of mean.

The abbreviation used is: ESMP = EDTA submitochondrial particle.

[a] Ten sham operated rats and jaundiced rats were utilized in this experiment.

[b] From 100 mg of mitochondria protein.

[c] $P<0.001$ compared with control groups.

**Legend for figure**

Fig. 1. Western blot with anti-bovine heart mitochondrial $F_1$ antibody. Lane a, molecular weight standards; lane b, purified bovine heart mitochondrial $F_1$ (0.2 μg protein); lane c, ESMP obtained from control rat liver at 1 hr after induction of hemorrhagic shock (10 μg protein); lane d, ESMP obtained from jaundiced rat liver at 1 hr after induction of hemorrhagic shock (10 μg protein). α: α subunit, β: β subunit. The molecular mass values for the standards are indicated on left in kilodalton. The α and β subunits are the two prominent bands observed between the 66.2 and 45 kilodalton standards.

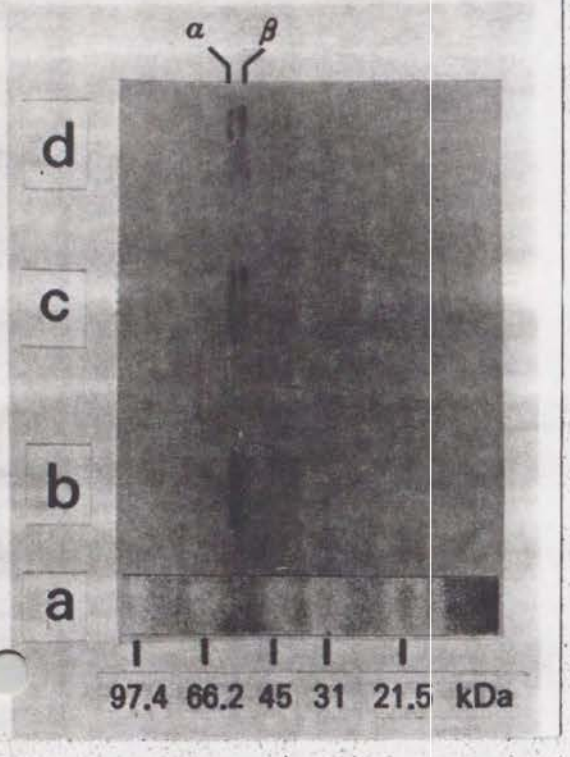


Fig 1